# Mechanistic prediction of Genotoxicity using Cell Painting

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## Introduction

Assessing genotoxicity is a regulatory requirement during drug development. Currently a battery of expensive low throughput assays are required to assess the multiple mechanisms in which genotoxicity can manifest. Improved predictive tools for assessing toxicity liabilities earlier in the drug development process are needed, to reduce the reliance on multiple animal studies and therefore the time and cost of development of new medicines.

Cell Painting (CP) is a high content, target agnostic screening assay. CP offers a significant advantage over traditional imaging assays which are limited to measuring single endpoints relating to genotoxicity, such as micronuclei formation, and do not consider other types of toxicity which may become apparent in the clinic.

In collaboration with Phenaros [1], a company based out of Uppsala University, specializing in AI driven phenomics we performed a pilot study aiming to develop novel predictive models of genotoxicity utilising data generated from CP.

## Methods

- An AI based tool was used to randomize the plate layouts in an effective manner [2]. Replicate assay ready plates, containing a library of 150 compounds with known genotoxicity annotations together with phenotypic reference controls were prepared using an Echo acoustic dispenser and distributed to AZ and Phenaros.
- A549 cells were dispensed into the pre-prepared compound containing plates, incubated for 48 hours then stained with six fluorescent dyes to label the different cellular compartments namely the nucleus, endoplasmic reticulum, mitochondria, cytoskeleton, Golgi apparatus, nucleoli and cytoplasmic RNA according to the protocol developed by the JUMP Cell Painting consortium [3]. Staining was performed at each site using a fully automated setup.
- Plates were imaged on a Yokogawa CV8000 confocal microscope at AZ and on a CEPHLA SQUID wide field microscope at Phenaros.
- Morphological features were extracted using CellProfiler, normalised and a feature selection process applied. The phenotypic strength of the compound perturbations was calculated using the grit score. Predictive models were then built using several machine learning methods.

## Results

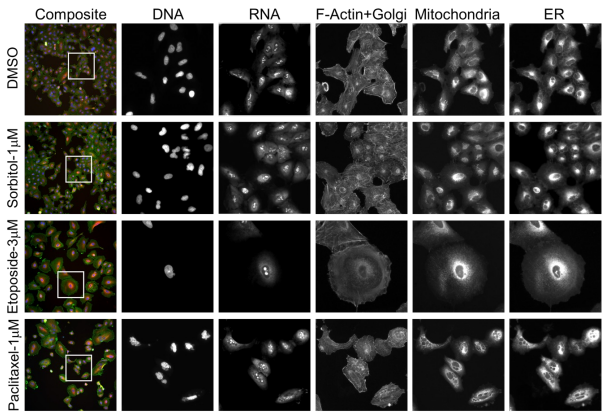


**Figure 1: Representative images of the phenotypic controls.** Images were obtained with the CEPHLA SQUID microscope using a 20x objective.

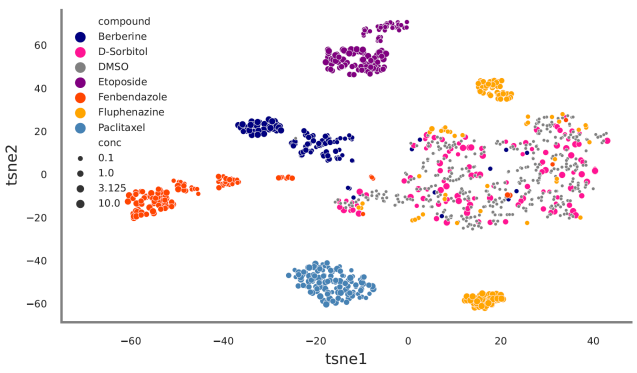


**Figure 2: tSNE Representation of the phenotypic reference controls.** Concentration is represented by the size of the markers. Clear separation and a dose dependant effect of compounds in morphological space can be observed. Controls with no known toxic effects cluster together with DMSO as expected.

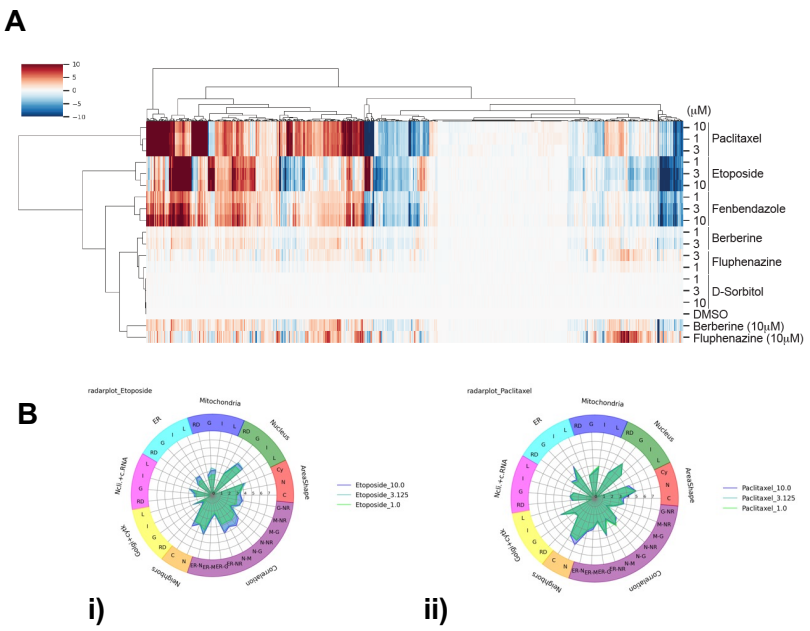


**Figure 3: Cell Painting signatures as a clustergram and radar plots.**
From the clustergram very distinct morphological signatures can be observed (A). Radar plots (B) are shown for the clastogenic (Etoposide-i) and aneugenic (Paclitaxel-ii) controls again showing distinct fingerprints.

Phenotypic controls showed clear visual differences (Fig. 1) which translated into separation in morphological space (Fig. 2). Clastogenic (Etoposide) and aneugenic (Paclitaxel) controls used in current routine genotoxicity assays exhibited very distinct CP signatures as shown in the clustergram and radar plots (Fig. 3 A/B).

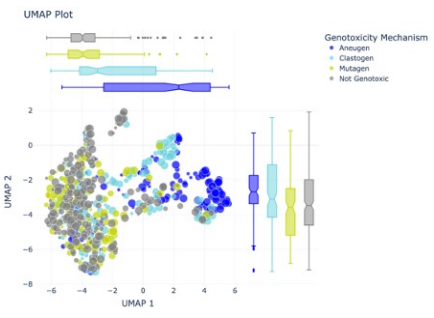


**Figure 4: UMAP analysis of** AZ generated data showing clear separation of aneugen and clastogens in morphological space. Mutagens however cannot be detected using this technique. Concentration is indicated by the size of the markers.

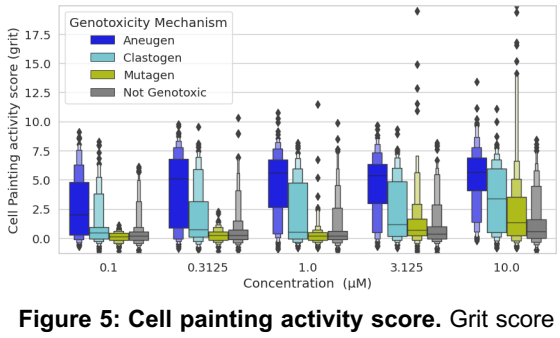


**Figure 5: Cell painting activity score.** Grit score for the three main genotoxicity mechanisms (aneugens, clastogens and mutagens) and the non-genotoxic compounds at concentrations.

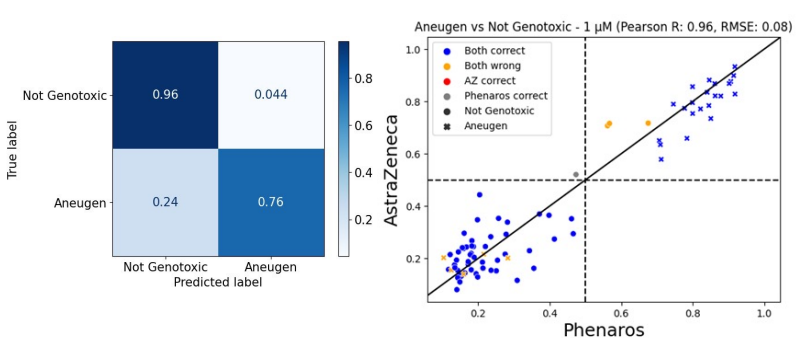


**Figure 6: Model Performance and Prediction Correlations between AstraZeneca and Phenaros using a Random Forest algorithm and data from 1uM**

Interestingly, based on morphology alone, aneugenic and clastogenic compounds displayed a distinct phenotype and separated from mutagenic and non-genotoxic compounds (Fig. 5). Predictive models were then built using several machine learning methods and results from these demonstrated that Cell Painting can be used to accurately predict compound genotoxicity at much lower compound doses than traditional assays and showed excellent reproducibility across the 2 test sites (Fig. 6).

## Conclusions

- We have demonstrated that Cell Painting can be used to predict compound genotoxicity and separate key mechanistic classes.
- Two independent experimental sites were able to produce high quality data and make mechanistic predications with a high degree of accuracy and reproducibility.
- Deploying Cell Painting early in the drug discovery process offers the potential to provide a holistic assessment of multiple toxicities, allowing a deeper mechanistic understanding of on and off target toxicity and therefore reducing our reliance on animal studies to assess the safety of new medicines.

**References**
1. www.phenaros.com
2. Rodriguez *et al* Artificial Intelligence in the life sciences, 3, (2023)
3. Cimini *et al.* Nature Protocols, 18 (7), (2023)

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